Infra-red Microspectral Classification of Cell Populations Under Different Growth Conditions, and Correlations with Genomic Expression Data

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INTRODUCTION

The biochemical state of a growing cell culture is a dynamic response of the organism's genetic instructions (genome sequence) regulating and expressing molecular (metabolic) products to control its structural and compositional state (epigenesis). Advances in microarray technology have now made it possible to capture a detailed profile of transcription levels at a given moment in time and provide a wealth of data to the biochemist. Integrating and understanding this data is a complex task and there is often a need to diagnose or classify the condition of cells (e.g. normal of cancerous) with more rapid and nondestructive methods. Microscopy has long served as a method to observe cell morphology and combination of microscopic spectral measurements have emerged as a technique for imaging and perhaps measuring physical characteristics such as overall composition (e.g. synchrotron microspectral infra-red techniques [1,2]).

EXPERIMENTAL

1. Biological Protocols

Mammalian cell cultures derived from mouse hepatoma cell lines were grown in conditions of deficient, excess and normal iron levels. Iron overload was induced in the cultured cells by incubation in a 100 ug/ml concentration of iron; for inducing iron deficiency, we used an iron chelator Dsferroxamine mesylate (DFO) at 75 uM levels. Treated cells were collected after 24 hours of development following exposure to the varying iron levels, including a control cell line. To investigate changes in gene expression during iron excess and overload in mouse we examined the gene expression pattern in nutritionally and genetically iron overloaded or deficient cells. cDNA microarray analysis was used to measure the relative abundance of genes expressed in each of these cell lines reverse transcription (using established LBNL protocols) of the entire mRNA pool with a flourescently labeled nucleotide (FluoroLink Cy3-dUTP, Amersham). A second probe labeled with a different color flourophor (FluoroLink Cy5-dUTP, Amersham) will be prepared from the control mRNA sample for comparison.

2. Spectroscopic Protocols

The mammalian cells were cultured on a gold coated microscope slide and air dried following rinsing and blot drying. Cells which adhered to the surface were then imaged using the beamline 1.4.3 FTIR microscope (Nicolet, Omnic software) and infra-red spectra measured in the reflectance mode, relative to a blank slide background. The spectra were analyzed following correction for background and baseline drift.

RESULTS

Significant differences in gene expression for the cell populations as shown in figure 1, which plots the hybrized fluorescence intensity ratios of the second probe (R = Fluorolink Cy5-dUTP) against the first probe (G = Fluorolink cy3-dUTP) for 8735 gene products.

The genes may be classified by a clustering algorithm (k-means by normal distance) to further show functional relationships between the genes and the biochemical pathways which they are involved in, as shown in figure 2.

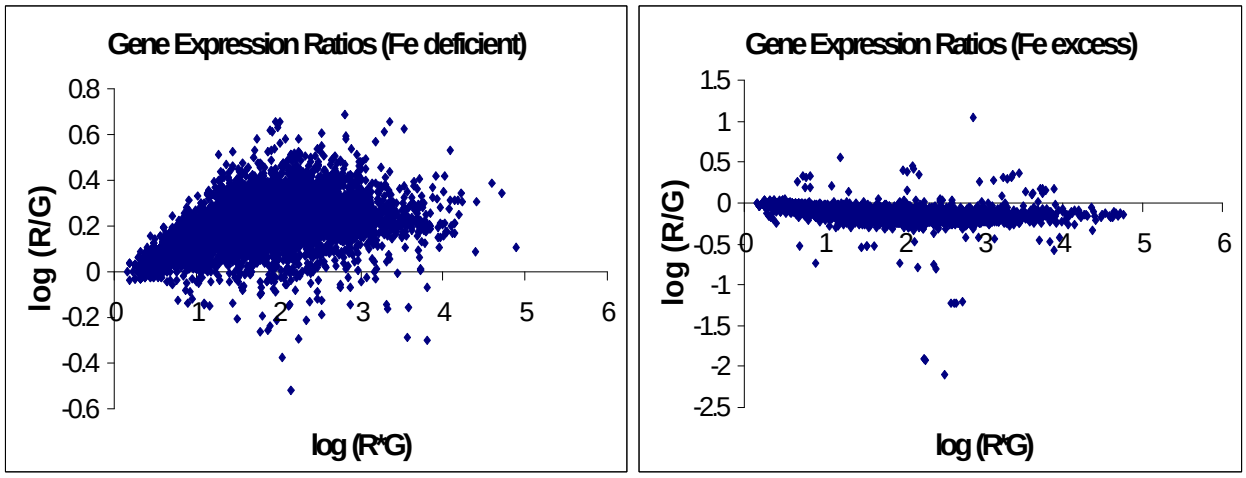

Figure 1. Expression levels of 8735 genes for hepatoma cells cultured under conditions of iron deficiency (75 uM DFO) and iron excess (100 ug/ml [Fe]). Significant differences versus the control show genes that may be involved in biochemical pathways related to Fe metabolism.

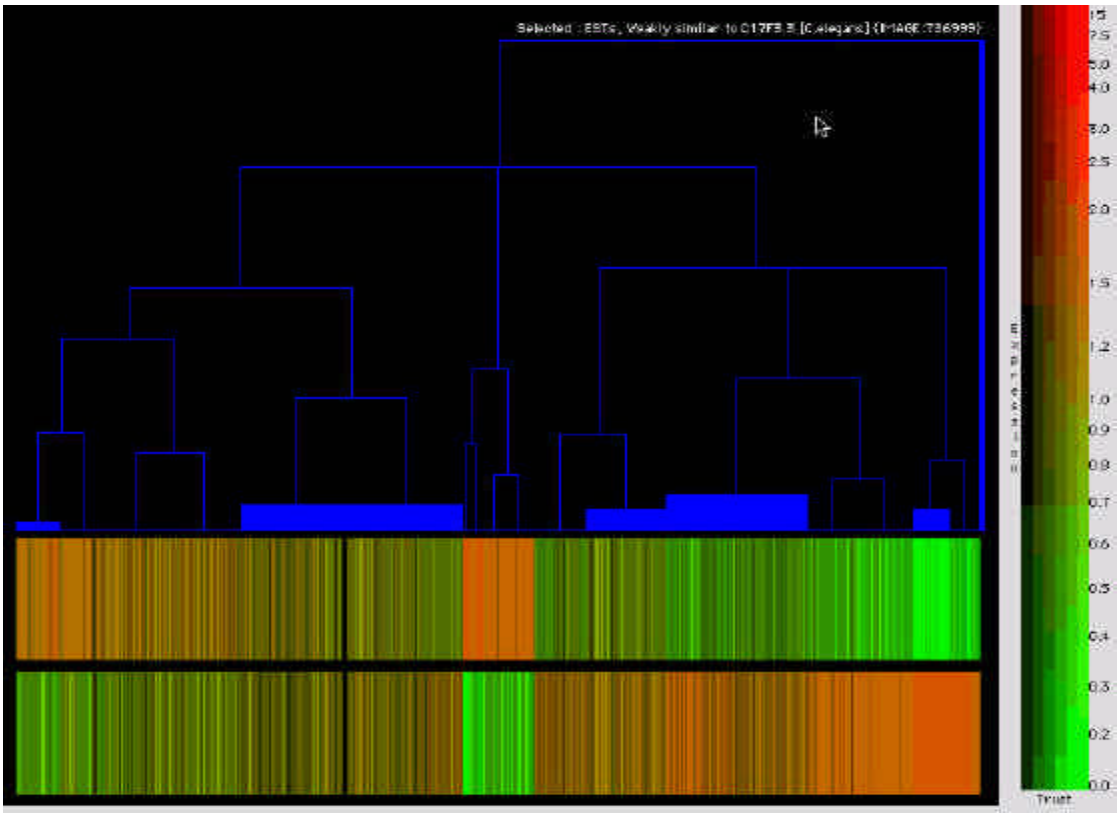

Figure 2. k-means clustering of ~800 genes showing notable correlated changes in expression level as a response to iron levels.

Vibrational spectra were acquired for live, individual cells by using the spatial discrimination powers of an FTIR microscope. The cells were mounted on a microscope slide, observed using white light microscopy and individual spectra were acquired by the Fourier transform infra-red absorption (taken on beamline 1.4.3, taking advantage of the bright synchrotron light source). Typical reflectance mode spectra are shown in figure 3 below.

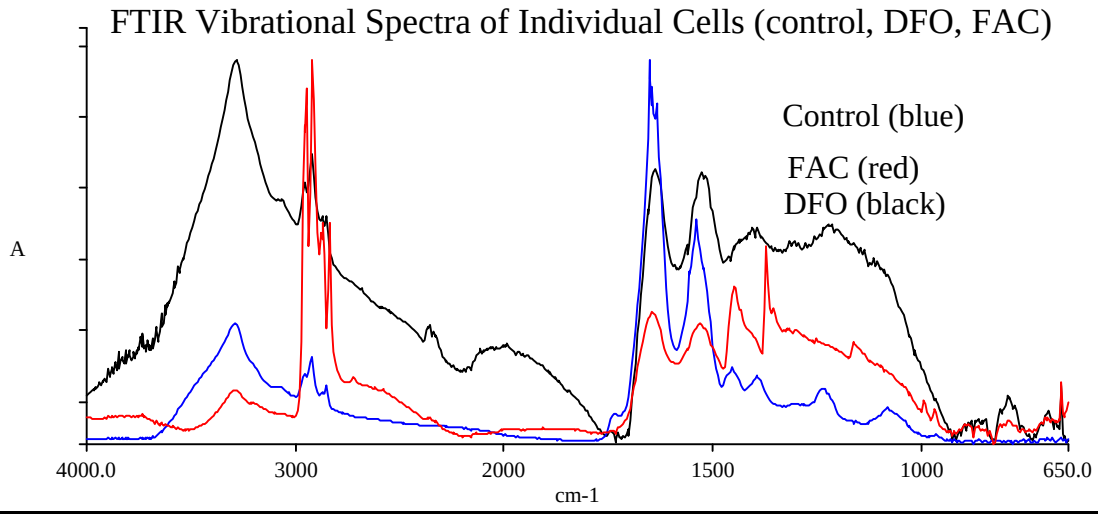

Figure 3. FTIR spectra of individual cells grown under different iron nutrition levels. The IR spectra are a complex superposition of contributions from all of the infra-red active materials present within the cell, although some distinct features such as the amide bands from proteins can be clearly recognized.

Since the FTIR spectra measure an intensity weighted sum of the molecular components which comprise the cell, biochemical consequences of regulated pathways may show characteristic signatures that could be correlated with the metabolic state of the cell. Through the use of mathematical analysis (principle components and other data mining techniques) performed on statistically significant cell numbers we hope to discover correlations between regulation of gene expression and more macroscopic compositional and structural changes that will be captured in the vibrational spectra.

REFERENCES

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